AMENDMENTS

Amendments to the Specification

1. Please replace paragraph 48 with the one below:

Another embodiment of the present invention provides a modified neurotoxin comprising a botulinum toxin (such as a botulinum toxin type A) which includes a structural modification which is effective to alter a biological persistence of the modified neurotoxin relative to an identical neurotoxin without the structural modification. The structural modification can comprise a deletion of amino acids 416 to 437 from a light chain of the neurotoxin ~~(Fig. 3)~~ of SEQ ID NO: 29.

2. Please replace paragraph 49 with the one below:

In still another embodiment of the present invention there is provided a modified neurotoxin (such as a botulinum toxin type A) which includes a structural modification which is effective to alter a biological persistence of the modified neurotoxin relative to an identical neurotoxin without the structural modification. The structural modification can comprise a deletion of amino acids 1 to 8 from a light chain of the neurotoxin ~~(Fig. 3)~~ of SEQ ID NO: 29.

3. Please replace paragraph 50 with the one below:

Still further in accordance with the present invention there is provided a modified neurotoxin, such as a botulinum toxin type A, which includes a structural modification which is effective to alter a biological persistence of the modified neurotoxin relative to an identical neurotoxin without the structural modification. The structural modification may comprise, for example, a deletion of 2 or more amino acids from 1 to 20 and a deletion of 2 or more amino acids from 398 to 437 from a light chain of the neurotoxin of SEQ ID NO: 29. In one embodiment, the structural modification comprises a deletion of amino acids 1 to 8 and 416 to 437 from a light chain of the neurotoxin ~~(Fig. 3)~~ of SEQ ID

NO: 29. In some embodiments, the structural modification comprises a deletion of amino acids 1 to 9 and 416 to 437 from a light chain of the neurotoxin of SEQ ID NO: 29. With regard to deletion on either the 1-8 or 1-9 amino acids; after synthesis the initial Methionine (M) of, for example, BoNT/A is apparently posttranslationally removed within Clostridia. Amino acids 1 – 8 do not include the initial Met residue. If one includes the initial Met residue, then amino acids 1 – 9 are removed. Of course a recombinant toxin would need a Met residue incorporated to start protein synthesis. It may or may not be removed following synthesis.

4. Please replace paragraph 51 with the one below:

For example, a native synthesized BoNT/A can comprise: MPFVNKQFNYKD (SEQ ID NO: 14), whereas a native processed BoNT/A can comprise PFVNKQFNYKD (SEQ ID NO: 15). Thus a proposed 8 amino acid deletion of SEQ ID NO: 27 would retain the YKD amino acid residues, while a recombinantly produced deletion would retain the ~~MYKD~~ amino acid residues of SEQ ID NO: 16 (MYKD).

5. Please replace paragraph 52 with the one below:

Still further in accordance with the present invention, there is provided a modified botulinum toxin, such as a modified botulinum toxin type A, which includes a structural modification effective to alter a biological persistence of the modified neurotoxin relative to an identical neurotoxin without said structural modification. The structural modification can comprise a substitution of leucine at position 427 for an alanine and a substitution of leucine at position 428 for an alanine in a light chain of said neurotoxin ~~(Fig. 3)~~ of SEQ ID NO: 29.

6. Please replace paragraph 72 with the one below:

Fig. 1 shows localization of GFP-botulinum toxin A light chain in (nerve growth factor) NGF-differentiated live PC12 cells visualized on a fluorescence inverted microscope.

The arrow indicates that GFP-botulinum toxin A light chain localizes to the plasma membrane.

7. Please replace paragraph 73 with the one below:

Fig. 2 shows the localization of GFP-truncated botulinum toxin A light chain in NGF-differentiated live PC12 cells visualized on a fluorescence inverted microscope. The arrow indicates that GFP-truncated botulinum toxin A light chain localizes to punctate bodies inside the cytoplasm.

8. Please replace paragraph 74 with the one below:

Fig. 3 shows the amino acid sequence for botulinum type A light chain. The amino acid sequence of SEQ ID NO: 29 shown, minus the underlined amino acids represents botulinum type A truncated light chain. The overline labeled ΔN8 indicates the eight amino acids deleted from the amino terminus of the light chain, the overline labeled ΔC22 indicates the 22 amino acids deleted from the carboxy terminus of the light chain. The double underline indicates the leucine-based motif and the dotted lines indicate tyrosine-based motifs.

9. Please replace paragraph 75 with the one below:

Fig. 4 shows the localization of GFP-botulinum toxin A light chain with LL to AA mutation at position 427 and 428 in NGF-differentiated live PC12 cells visualized on a fluorescence inverted microscope. The arrow indicates that GFP-botulinum toxin A light chain with LL to AA mutation localizes to punctate bodies inside the cytoplasm.

10. Please replace paragraph 76 with the one below:

Fig. 5 shows localization of fluorescently labeled anti-SNAP-25 visualized in horizontal confocal sections of staurosporine-differentiated PC12 cells. The arrow indicates that SNAP-25 localizes to the plasma membrane.

11. Please replace paragraph 78 with the one below:

Fig. 7 shows localization of GFP-botulinum type B neurotoxin light chain in NGF-differentiated live PC12 cells visualized on a fluorescence inverted microscope. The arrow indicates that GFP-botulinum toxin B light chain localizes to punctate bodies inside the cytoplasm.

12. Please replace paragraph 79 with the one below:

Fig. 8 shows sequence alignment and consensus sequence for botulinum toxin type A Hall A light chain of SEQ ID NO: 29 and botulinum toxin type B Danish I light chain of SEQ ID NO: 30.

13. Please replace paragraph 81 with the one below:

Fig. 10 shows a comparison of LC/A constructs expressed from E. coli for in vitro analysis. The LC/A (WT) sequences shown are amino acids 2-14 of SEQ ID NO: 29 (Amino terminus) and amino acids 412-438 of SEQ ID NO: 29 (Carboxyl Terminus). The LC/A (ΔN8/ΔC22) sequences shown are SEQ ID NO: 25 (Amino terminus) and SEQ ID NO: 26 (Carboxyl Terminus). The N-His LC/A (WT) sequences shown are SEQ ID NO: 148 (Amino terminus) and amino acids 412-438 of SEQ ID NO: 29 (Carboxyl Terminus).

14. Please replace paragraph 91 with the one below:

Fig. 20 shows activity assessed by western blot of the lysate of ~~cells transfected with GFP, GFP-LCA, GFP-LCE, and GFP+LCA~~ transfected cells. Fig 20A shows the presence of the SNAP-25_{197} BoNT/A cleavage product in lysates containing GFP-LCA and GFP + LCA, but not GFP alone. Fig. 20B shows the presence of the SNAP-25_{180} BoNT/E cleavage product in lysates containing GFP-LCE, but not GFP alone.

15. Please replace paragraph 92 with the one below:

Fig. 21 shows that light chain A localizes to the plasma membrane. The top panel shows that GFP alone exhibits a diffuse cytoplasmic localization. However, the bottom panel shows that GFP-botulinum toxin A light chain localizes to the plasma membrane.

16. Please replace paragraph 93 with the one below:

Fig. 22 shows that light chain B localizes in the cytoplasm. The top panel shows that GFP-botulinum toxin B light chain exhibits a diffuse cytoplasmic localization. The bottom panel shows that botulinum toxin B light chain-GFP localizes to punctate bodies inside the cytoplasm.

17. Please replace paragraph 94 with the one below:

Fig. 23 shows that Light Chain E also localizes primarily in the cytoplasm. The top panel shows that GFP-botulinum toxin E light chain exhibits a semi-diffuse cytoplasmic localization. The bottom panel shows that botulinum toxin B light chain-GFP exhibits a diffuse cytoplasmic localization.

18. Please replace paragraph 98 with the one below:

Fig. 27 shows localization of Light Chains in HeLa is similar to PC12 Cells. The panel on the left shows that GFP-botulinum toxin A light chain localizes to the plasma membrane. The middle panel shows that GFP-botulinum toxin B light chain exhibits a diffuse cytoplasmic localization. The panel on the right shows that GFP-botulinum toxin E light chain exhibits a semi-diffuse cytoplasmic localization.

19. Please replace paragraph 100 with the one below:

Fig. 29 shows HEK293T cells transfected with plasmids encoding GFP-LCA, GFP-LCE, GFP-LCB, and LCB-GFP. The panel on the left shows that GFP-botulinum toxin A light chain localizes to the plasma membrane. The middle panel shows that GFP-botulinum

toxin B light chain exhibits a diffuse cytoplasmic localization. The panel on the right shows that GFP-botulinum toxin E light chain exhibits a semi-diffuse cytoplasmic localization.

20. Please replace paragraph 113 with the one below:

In one embodiment, the leucine-based motif is xDxxxLL (SEQ ID NO: 17), wherein x can be any amino acids. In another embodiment, the leucine-based motif is xExxxLL (SEQ ID NO: 18), wherein E is glutamic acid. In another embodiment, the duplet of amino acids can include an isoleucine or a methionine, forming xDxxxLI (SEQ ID NO: 19) or xDxxxLM (SEQ ID NO: 20), respectively. Additionally, the aspartic acid, D, can be replaced by a glutamic acid, E, to form xExxxLI (SEQ ID NO: 21), xExxxIL (SEQ ID NO: 22) and xExxxLM (SEQ ID NO: 23). In a preferred embodiment, the leucine-based motif is phenylalanine-glutamate-phenylalanine-tyrosine-lysine-leucine-leucine, ~~SEQID #1~~ SEQ ID NO: 1.

21. Please replace paragraph 140 with the one below:

Tyrosine-based motifs are within the scope of the present invention as biological persistence and/or a biological activity altering components. Tyrosine-based motifs comprise the sequence Y-X-X-Hy (SEQ ID NO: 24), where Y is tyrosine, X is any amino acid and Hy is a hydrophobic amino acid. Tyrosine-based motifs can act in a manner that is similar to that of leucine-based motifs. In figure 3 some of tyrosine motifs found in the type A toxin light chain are bracketed (SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, and SEQ ID NO: 38). In addition, a tyrosine-based motif is found within the leucine-based motif which is indicated by an asterisked bracket in figure 3.

22. Please replace paragraph 143 with the one below:

Figure 8 shows a sequence alignment between type A and type B light chains isolated from strains type A HallA (~~SEQ ID NO: 19~~SEQ ID NO: 29) and type B Danish I (~~SEQ ID~~

~~NO: 20~~SEQ ID NO: 30) respectively. Light chains or heavy chains isolated from other strains of botulinum toxin types A and B can also be used for sequence comparison. The shaded amino acids represent amino acid identities, or matches, between the chains. Each of the shaded amino acids between amino acid position 10 and amino acid position 425 of the Fig. 8 consensus sequence, alone or in combination with any other shaded amino acid or amino acids, represents a biological persistence altering component that is within the scope of the present invention. For example, amino acids KAFK at positions 19 to 22 of SEQ ID NO: 29, LNK at positions 304 to 306 of SEQ ID NO: 29, L at position 228 of SEQ ID NO: 29 in combination with KL at positions 95 and 96 of SEQ ID NO: 29, FDKLYK at positions 346 to 351 of SEQ ID NO: 29, YL-T at positions 78 to 81 of SEQ ID NO: 29, YYD at positions 73 to 75 of SEQ ID NO: 29 in combination with YL at positions 78 and 79 of SEQ ID NO: 29 in combination with T a position 81 of SEQ ID NO: 29, F at position 297 of SEQ ID NO: 29 in combination with I at position 300 of SEQ ID NO: 29 in combination with KL at positions 95 and 96 of SEQ ID NO: 29 can be biological persistence altering components for use within the scope of this invention. In addition, conserved regions of charge, hydrophobicity, hydro-philicity and/or conserved secondary, tertiary, or quaternary structures that may be independent of conserved sequence are within the scope of the present invention.

23. Please replace paragraph 275 with the one below:

Additional studies showed that a GFP-LCA construct with the eight amino acid residues of SEQ ID NO: 27 (PFVNKQFN) deleted from the N-terminus (no C-terminus deletion) localized in PC12 cells a very similar pattern to the localization in PC12 cells of a truncated GFP-LCA construct with both the C and N terminus deletions.

24. Please replace paragraph 276 with the one below:

Further studies showed that a GFP-LCA construct with the twenty two amino acid residues of SEQ ID NO: 28 (KNFTGLFEFYK**LL**CVRGIITSK) deleted from the C-terminus (no N-terminus deletion) localized in PC12 cells in a very similar manner to that of the GFP-LCA(LL-->AA) mutant.

25. Please replace paragraph 277 with the one below:

A GFP-LCA construct with both the eight amino acid residues of SEQ ID NO: 27 (PFVNKQFN) deleted from the N-terminus and the twenty two amino acid residues of SEQ ID NO: 28 (KNFTGLFEFYK**LL**CVRGIITSK) deleted from the C-terminus accumulated intracellularly.

26. Please replace paragraph 287 with the one below:

It has been observed that a recombinant construct with both the eight amino acid residues of SEQ ID NO: 27 (PFVNKQFN) deleted from the N-terminus and the twenty-two amino acid residues of SEQ ID NO: 28 (KNFTGLFEFYK**LL**CVRGIITSK) deleted from the C-terminus of the light chain of botulinum toxin A exhibits a reduced activity such that the effective concentration (EC_{50}) required to cleave the SNAP-25 substrate is nearly ten-fold greater than that of a similar construct with only the C-terminal twenty-two amino acid deletion (EC_{50} ΔN8ΔC22 =4663 pM *vs*. EC_{50}Δ C22 =566 pM). The recombinant light chain of botulinum toxin A was used as a control (EC_{50} rLC/A =7 pM), and, therefore, as compared to the rLC/A construct, a 666-fold greater concentration of the ΔNΔ8C22 construct is required. A recombinant light chain construct with the dileucine motif mutated to dialanine [rLC/A(LL-->AA)] also exhibits reduced activity (EC_{50} rLC/A(LL-->AA) =184 pM); however, the effective concentration of the ΔN8ΔC22 construct is twenty-five fold greater than the rLC/A(LL-->AA) construct.

27. Please replace paragraph 289 with the one below:

A chimeric botulinum toxin can be constructed such that a C-terminal portion of the light chain of one botulinum toxin serotype replaces a similar C-terminal portion within the light chain of another botulinum toxin serotype. For example, the last twenty two amino acid residues bearing the dileucine motif from the C-terminus of the light chain of BoNT/A can replace the last twenty two amino acid residues of the C-terminus of the light chain of BoNT/E. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPKINSFNYNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDF
HPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKA
NPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNI
SLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHELIHSLHG
LYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNL
LADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLDKDASGIYSVNINKFNDIFKK
LYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNINNLKVNFR
GQNANLNPRIITPITG**KNFTGLFEFYKLLCVRGIITSK** ~~(SEQ ID #63)~~ SEQ ID NO: 136

28. Please replace paragraph 291 with the one below:

In a further example, the first thirty amino acid residues from the N-terminus of the light chain of BoNT/A can replace the first thirty amino acid residues of the N-terminus of the light chain of BoNT/B. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPFVNKQFNYKDPVNGVDIAYIKIPNAGQMGRYYKAFKITDRIWIIPERYTFGYK
PEDFNKSSGIFNRDVCEYYDPDYLNTNDKKNIFFQTLIKLFNRIKSKPLGEKLLE
MIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNPGEVERKKGIFANLIIFGPGP
VLNENETIDIGIQNHFASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGY
FSDPALILMHELIHVLHGLYGIKVDDLPIVPNEKKFFMQSTDTIQAEELYTFGGQ
DPSIISPSTDKSIYDKVLQNFRGIVDRLNKVLVCISDPNININIYKNKFKDKYKF
VEDSEGKYSIDVESFNKLYKSLMLGFTEINIAENYKIKTRASYFSDSLPPVKIKN
LLDNEIYTIEEGFNISDKNMGKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSV
K ~~(SEQ ID #64)~~ SEQ ID NO: 137

29. Please replace paragraph 293 with the one below:

Still further, the chimeric construct can have both N-terminal and the C-terminal replacements. For example, the first nine amino acid residues from the N-terminus of the light chain of BoNT/A can replace the first nine amino acid residues of the N-terminus of the light chain of BoNT/E. Additionally, in the same construct, the last twenty-two amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last twenty-two amino acid residues from the C-terminus of the light chain of BoNT/E. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPFVNKQFNNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDF
HPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKA
NPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNI
SLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHELIHSLHG
LYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNL
LADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLDKDASGIYSVNINKFNDIFKK
LYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNINNLKVNFR
GQNANLNPRIITPITG**KNFTGLFEFYKLLCVRGIITSK**~~ (SEQ ID #65)~~ SEQ
ID NO: 138

30. Please replace paragraph 295 with the one below:

Similarly, the first nine amino acid residues from the N-terminus of the light chain of BoNT/A can replace the first nine amino acid residues of the N-terminus of the light chain of BoNT/B. Additionally, in the same construct, the last twenty-two amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last twenty-two amino acid residues from the C-terminus of the light chain of BoNT/B. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPFVNKQFNYNDPIDNDNIIMMEPPFARGTGRYYKAFKITDRIWIIPERYTFGYK
PEDFNKSSGIFNRDVCEYYDPDYLNTNDKKNIFFQTLIKLFNRIKSKPLGEKLLE
MIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNPGEVERKKGIFANLIIFGPGP
VLNENETIDIGIQNHFASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGY
FSDPALILMHELIHVLHGLYGIKVDDLPIVPNEKKFFMQSTDTIQAEELYTFGGQ
DPSIISPSTDKSIYDKVLQNFRGIVDRLNKVLVCISDPNININIYKNKFKDKYKF
VEDSEGKYSIDVESFNKLYKSLMLGFTEINIAENYKIKTRASYFSDSLPPVKIKN
LLDNEIYTIEEGFNISDKNMGKEYRGQNKAINKQ**KNFTGLFEFYKLLCVRGIITS**
K~~ (SEQ ID #66)~~ SEQ ID NO: 139

31. Please replace paragraph 297 with the one below:

Furthermore, the first nine amino acid residues from the N-terminus of the light chain of BoNT/A can replace the first nine amino acid residues of the N-terminus of the light chain of BoNT/F. Additionally, in the same construct, the last twenty-two amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last twenty-

two amino acid residues from the C-terminus of the light chain of BoNT/F. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPFVNKQFNYNDPVNDDTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTIGTN
PSDFDPPASLKNGSSAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGKVLLQE
ISYAKPYLGNDHTPIDEFSPVTRTTSVNIKLSTNVESSMLLNLLVLGAGPDIFES
CCYPVRKLIDPDVVYDPSNYGFGSINIVTFSPEYEYTFNDISGGHNSSTESFIAD
PAISLAHELIHALHGLYGARGVTYEETIEVKQAPLMIAEKPIRLEEFLTFGGQDL
NIITSAMKEKIYNNLLANYEKIATRLSEVNSAPPEYDINEYKDYFQWKYGLDKNA
DGSYTVNENKFNEIYKKLYSFTESDLANKFKVKCRNTYFIKYEFLKVPNLLDDDI
YTVSEGFNIGNLAVNNRGQSIKLNPKIID**KNFTGLFEFYKLLCVRGIITSK** ~~(SEQ ID #67)~~ SEQ ID NO: 140

32. Please replace paragraph 299 with the one below:

In some embodiments, a light chain can be engineered such that one or more segments of the light chain of one or more toxin serotypes replace one or more segments of equal or unequal length within the light chain of another toxin serotype. In a non-limiting example of this kind of chimeric construct, fifty amino acid residues from the N-terminus of the light chain of BoNT/A can replace eight amino acid residues of the N-terminus of the light chain of BoNT/B, resulting in a net gain of ~~fourty-two~~ forty-two amino acids in length in the N-terminal region of the light chain chimera. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

M**PFVNKQFNYKDPVNGVDIAYIKIPNAGQMQPVKAFKIHNKIWVIPERDTF**YNDP
IDNDNIIMMEPPFARGTGRYYKAFKITDRIWIIPERYTFGYKPEDFNKSSGIFNR
DVCEYYDPDYLNTNDKKNIFFQTLIKLFNRIKSKPLGEKLLEMIINGIPYLGDRR
VPLEEFNTNIASVTVNKLISNPGEVERKKGIFANLIIFGPGPVLNENETIDIGIQ
NHFASREGFGGIMQMKFCPEYVSVFNNVQENKGASIFNRRGYFSDPALILMHELI
HVLHGLYGIKVDDLPIVPNEKKFFMQSTDTIQAEELYTFGGQDPSIISPSTDKSI
YDKVLQNFRGIVDRLNKVLVCISDPNININIYKNKFKDKYKFVEDSEGKYSIDVE
SFNKLYKSLMLGFTEINIAENYKIKTRASYFSDSLPPVKIKNLLDNEIYTIEEGF
NISDKNMGKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSVK ~~(SEQ ID #68)~~
SEQ ID NO: 141

33. Please replace paragraph 301 with the one below:

In a non-limiting example of this kind of chimeric construct, the last fifty amino acid residues from the C-terminus of the light chain of BoNT/A can replace fifteen amino acid residues within the C-terminus of the light chain of BoNT/E, resulting in a net gain of thirty-five amino acids in the C-terminal region of the light chain chimera. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

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MPKINSFNYNDPVNDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIGTTPQDF
HPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILLEELSKA
NPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFETNSSNI
SLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHELIHSLHG
LYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSNDIYTNL
LADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLDKDASGIYSVNINKFNDIFKK
LYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNINNLKVNFR
GQNANLNPRIITPGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLC
VRGIITSKNIVSVKGIRK(SEQ ID #69) SEQ ID NO: 142
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34. Please replace paragraph 303 with the one below:

In a non-limiting example of this kind of chimeric construct, thirty amino acid residues from the N-terminus of the light chain of BoNT/A can replace ten amino acid residues of the N-terminus of the light chain of BoNT/E, resulting in a net gain of twenty amino acids in length in the N-terminal region of the chimera. Additionally, in the same construct, the last fifty amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last fifty amino acid residues from the C-terminus of the light chain of BoNT/E. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

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MPKINSFNYMPFVNKQFNYKDPVNGVDIAYIKIPNAGQMYIKPGGCQEFYKSFNI
MKNIWIIPERNVIGTTPQDFHPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKI
FNRINNNLSGGILLEELSKANPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILL
PNVIIMGAEPDLFETNSSNISLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMN
EFIQDPALTLMHELIHSLHGLYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTF
GGTDLNIITSAQSNDIYTNLLADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLD
KDASGIYSVNINKFNDIFKKLYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLN
DSIYNISEGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYKLLCVRGII
TSK(SEQ ID #70) SEQ ID NO: 143
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35. Please replace paragraph 305 with the one below:

In a non-limiting example of this kind of chimeric construct, thirty amino acid residues from the N-terminus of the light chain of BoNT/A can replace ten amino acid residues of the N-terminus of the light chain of BoNT/B, resulting in a net gain of twenty amino acids in length in the N-terminal region of the chimera. Additionally, in the same construct, the last fifty amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last fifty amino acid residues from the C-terminus of the light chain of BoNT/B. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPVTINNFN**MPFVNKQFNYKDPVNGVDIAYIKIPNAGQM**IMMEPPFARGTGRYYK
AFKITDRIWIIPERYTFGYKPEDFNKSSGIFNRDVCEYYDPDYLNTNDKKNIFFQ
TLIKLFNRIKSKPLGEKLLEMIINGIPYLGDRRVPLEEFNTNIASVTVNKLISNP
GEVERKKGIFANLIIFGPGPVLNENETIDIGIQNHFASREGFGGIMQMKFCPEYV
SVFNNVQENKGASIFNRRGYFSDPALILMHELIHVLHGLYGIKVDDLPIVPNEKK
FFMQSTDTIQAEELYTFGGQDPSIISPSTDKSIYDKVLQNFRGIVDRLNKVLVCI
SDPNININIYKNKFKDKYKFVEDSEGKYSIDVESFNKLYKSLMLGFTEINIAENY
KIKTRASYFSDSLPPVKIKNLLDNEI**GFNLRNTNLAANFNGQNTEINNMNFTKLK**
NFTGLFEFYKLLCVRGIITSK~~(SEQ ID #71)~~ SEQ ID NO: 144

36. Please replace paragraph 307 with the one below:

In a non-limiting example of this kind of chimeric construct, thirty amino acid residues from the N-terminus of the light chain of BoNT/A can replace ten amino acid residues of the N-terminus of the light chain of BoNT/F, resulting in a net gain of twenty amino acids in length in the N-terminal region of the chimera. Additionally, in the same construct, the last fifty amino acid residues from the C-terminus of the light chain of BoNT/A can replace the last fifty amino acid residues from the C-terminus of the light chain of BoNT/F. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

MPVAINSFN**MPFVNKQFNYKDPVNGVDIAYIKIPNAGQM**LYMQIPYEEKSKKYYK
AFEIMRNVWIIPERNTIGTNPSDFDPPASLKNGSSAYYDPNYLTTDAEKDRYLKT
TIKLFKRINSNPAGKVLLQEISYAKPYLGNDHTPIDEFSPVTRTTSVNIKLSTNV
ESSMLLNLLVLGAGPDIFESCCYPVRKLIDPDVVYDPSNYGFGSINIVTFSPEYE

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YTFNDISGGHNSSTESFIADPAISLAHELIHALHGLYGARGVTYEETIEVKQAPL
MIAEKPIRLEEFLTFGGQDLNIITSAMKEKIYNNLLANYEKIATRLSEVNSAPPE
YDINEYKDYFQWKYGLDKNADGSYTVNENKFNEIYKKLYSFTESDLANKFKVKCR
NTYFIKYEFLKVPNLLDDDIYGFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGL
FEFYKLLCVRGIITSK (SEQ ID #72) SEQ ID NO: 145
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37. Please replace paragraph 309 with the one below:

In some embodiments, the swapped sequences can be derived from two different serotypes, resulting in a chimera with regions from three different serotypes in all. In this example, eight amino acid residues from the N-terminus of the light chain of BoNT/B can replace five amino acid residues of the N-terminus of the light chain of BoNT/E, resulting in a net gain of three amino acids in length in the N-terminal region of the chimera. Additionally, in the same construct, 30 amino acid residues including the dileucine repeat of the C-terminus of the light chain of BoNT/A can replace ten amino acid residues within the C-terminus of the light chain of BoNT/E, resulting in a net gain of 20 amino acids in the C-terminal region of the chimera. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

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MPKINSFNYNDPVTINNFNYDRTILYIKPGGCQEFYKSFNIMKNIWIIPERNVIG
TTPQDFHPPTSLKNGDSSYYDPNYLQSDEEKDRFLKIVTKIFNRINNNLSGGILL
EELSKANPYLGNDNTPDNQFHIGDASAVEIKFSNGSQDILLPNVIIMGAEPDLFE
TNSSNISLRNNYMPSNHGFGSIAIVTFSPEYSFRFNDNSMNEFIQDPALTLMHEL
IHSLHGLYGAKGITTKYTITQKQNPLITNIRGTNIEEFLTFGGTDLNIITSAQSN
DIYTNLLADYKKIASKLSKVQVSNPLLNPYKDVFEAKYGLDKDASGIYSVNINKF
NDIFKKLYSFTEFDLATKFQVKCRQTYIGQYKYFKLSNLLNDSIYNISEGYNINN
LKVNFRGQNANLNPRIITPITGRGLVKKIIRFCKNNMNFTKLKNFTGLFEFYKLL
CVRGIITSK (SEQ ID #73) SEQ ID NO: 146
```

38. Please replace paragraph 311 with the one below:

In a non-limiting example, eight amino acid residues from the N-terminus of the light chain of BoNT/B can replace five amino acid residues of the N-terminus of the light chain of BoNT/F, resulting in a net gain of three amino acids in length in the N-terminal region of the chimera. Additionally, in the same construct, 30 amino acid residues including the dileucine repeat of the C-terminus of the light chain of BoNT/A can replace ten amino

acid residues within the C-terminus of the light chain of BoNT/F, resulting in a net gain of 20 amino acids in the C-terminal region of the chimera. The amino acid sequence of the entire light chain of such a chimeric construct is shown below:

```
MPVAINSFNYNDVTINNFNYTILYMQIPYEEKSKKYYKAFEIMRNVWIIPERNTI
GTNPSDFDPPASLKNGSSAYYDPNYLTTDAEKDRYLKTTIKLFKRINSNPAGKVL
LQEISYAKPYLGNDHTPIDEFSPVTRTTSVNIKLSTNVESSMLLNLLVLGAGPDI
FESCCYPVRKLIDPDVVYDPSNYGFGSINIVTFSPEYEYTFNDISGGHNSSTESF
IADPAISLAHELIHALHGLYGARGVTYEETIEVKQAPLMIAEKPIRLEEFLTFGG
QDLNIITSAMKEKIYNNLLANYEKIATRLSEVNSAPPEYDINEYKDYFQWKYGLD
KNADGSYTVNENKFNEIYKKLYSFTESDLANKFKVKCRNTYFIKYEFLKVPNLLD
DDIYTVSEGFNIGNLAVNNRGQSIKLNPKIIDSIPDKGLVEKNNMNFTKLKNFTG
LFEFYKLLCVRGIITSKRK(SEQ ID #74) SEQ ID NO: 147
```

39. Please replace Table 2 with the one below:

Table 2				
Toxin ~~toxin~~	N-term (AAs 1-30) of LC	SEQ ID NO:	C-term (last 50 AAs) of LC	SEQ ID NO: ~~Seq ID #~~
BoNT/A	MPFVNKQFNYKDPVNGVDI AYIKIPNAGQM	39	GFNLRNTNLAANFNGQNTE INNMNFTKLKNFTGLFEFY KLLCVRGIITSK	~~14~~40
BoNT/B	MPVTINNFNYNDPIDNDNI IMMEPPFARGT	41	YTIEEGFNISDKNMGKEYR GQNKAINKQAYEEISKEHL AVYKIQMCKSVK	~~15~~42
BoNT/C_1	MPITINNFNYSDPVDNKNI LYLDTHLNTLA	43	NIPKSNLNVLFMGQNLSRN PALRKVNPENMLYLFTKFC HKAIDGRSLYNK	~~16~~44
BoNT/D	MTWPVKDFNYSDPVNDNDI LYLRIPQNKLI	45	YTIRDGFNLTNKGFNIENS GQNIERNPALQKLSSESVV DLFTKVCLRLTK	~~17~~46
BoNT/E	MPKINSFNYNDPVNDRTIL YIKPGGCQEFY	47	GYNINNLKVNFRGQNANLN PRIITPITGRGLVKKIIRF CKNIVSVKGIRK	~~18~~48
BoNT/F	MPVAINSFNYNDPVNDDTI LYMQIPYEEKS	49	TVSEGFNIGNLAVNNRGQS IKLNPKIIDSIPDKGLVEK IVKFCKSVIPRK	~~19~~50
BoNT/G	MPVNIKNFNYNDPINNDDI IMMEPFNDPGP	51	QNEGFNIASKNLKTEFNGQ NKAVNKEAYEEISLEHLVI YRIAMCKPVMYK	~~20~~52

40. Please replace Table 3 with the one below:

Table 3				
Toxin **~~toxin~~**	**N-term (AAs 1-30) of LC**	**SEQ ID NO:**	**C-term (last 50 AAs) of LC**	**SEQ ID NO:** **~~Seq ID #~~**
BoNT/A	MPF**A**NKQFNYKDPVNGVDI AYIKIPNAGQM	53	GFNLRNTNLAANFNGQNTE INNMN**R**TKLKNFTGLFEFY KLLCVRGIITSK	~~21~~54
BoNT/A	MPFVNKQFN**K**KDPVNGVDI AYIKIPNAGQM	55	GFNLRNTNLAANFNGQNTE INNMNFTKLKN**AA**GLFEFY KLLCVRGIITSK	~~22~~56
BoNT/A	MPFVNKQFNYKDPVNGVDI A**R**IKIPNAGQM	57	GFNLRNTNLAAN**H**NGQNTE INNMNFTKLKNFTGLFEFY KLLCVRGIITSK	~~23~~58
BoNT/A	MPFVNK**H**FNYKDPVNGVDI AYIKIPNAGQM	59	GFNLRNTNLAANFNGQNTE INNMNFTKLKNFTGLFEFY KLLC**A**RGIITSK	~~24~~60
BoNT/B	MP**A**TINNFNYNDPIDNDNI IMMEPPFARGT	61	YTIEEGFNISDKNMGKEYR GQNKAINKQAYEEISKEHL AVYKI**R**MCKSVK	~~25~~62
BoNT/B	MPVTINNFNYNDPIDNDNI I**AA**EPPFARGT	63	YTIEEGFNISDKNMGKEYR GQNKAINKQAYEEISKEHL AV**R**KIQMCKSVK	~~26~~64
BoNT/B	MPVTINNFN**R**NDPIDNDNI IMMEPPFARGT	65	YTIEEGFNISDKNMGKEYR GQNKAINKQA**K**EEISKEHL AVYKIQMCKSVK	~~27~~66
BoNT/C_1	MPITINN**K**NYSDPVDNKNI LYLDTHLNTLA	67	NIPKSNLNVLFMGQNLSRN PALRKVNPENMLYLFTKFC HKAIDGRSL**R**NK	~~28~~68
BoNT/D	MTWP**A**KDFNYSDP**A**NDNDI LYLRIPQNKLI	69	YTIRDGFNLTNKGFNIENS GQNIERNPALQKLSSESVV DLFTK**A**CLRLTK	~~29~~70
BoNT/E	MPKINSFNYNDP**A**NDRTIL YIKPGGCQEFY	71	GYNINNLKVNFRGQNANLN PRIITPITGRG**H**VKKIIRF CKNIVSVKGIRK	~~30~~72
BoNT/E	MPKINS**R**NYNDPVNDRTIL YIKPGGCQEFY	73	GYNINNLKVNFRGQNANLN PRIITPITGRGLVKKIIRF CKN**AA**SVKGIRK	~~31~~74
BoNT/E	MPKINSFNYNDPVNDRTIL YIKPGGCQEF**R**	75	GYNINNLKVNFRGQNANLN PRIITPITGRGLVKKIIRF	~~32~~76

			CKNIVS**A**KGIRK	
BoNT/F	MP**A**AINSFNYNDPVNDDTI LYMQIPYEEKS	77	TVSEGFNIGNLAVNNRGQS IKLNPKIIDSIPDKGLVEK IVKFCKS**A**IPRK	~~33~~78
BoNT/G	MPVNIKN**H**NYNDPINNDDI IMMEPFNDPGP	79	QNEGFNIASKNLKTEFNGQ NKAVNKEAYEEISLEHLVI YRIAMCKP**A**MYK	~~34~~80

41. Please replace Table 4 with the one below:

Table 4

Toxin ~~**toxin**~~	**N-term (AAs 1-30) of LC**	**SEQ ID NO:**	**C-term (last 50 AAs) of LC**	**SEQ ID NO:** ~~**Seq ID #**~~
BoNT/A	MPFVNKQFNYKDPVNGVDIAYIKIP**H**----	81	GFNLRNTNLAANFNGQNTEINNMN**AAAAAAAAAA**-------CVRGIITSK	~~35~~82
BoNT/A	M**AAA**----NYKDPVNGVDIAYIKIPNAGQM	83	G**K**NLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYK—CVRGIITSK	~~22~~84
BoNT/A	MPFVNKQFNYKDPVNGVDIA**R**----NAGQM	85	GFNLRNTNLAA----**H**NTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK	~~23~~86
BoNT/A	MP**K**VNKQFN----VNGVDIAYIKIPNAGQM	87	GFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEF**RR**--------TSK	~~24~~88
BoNT/B	MPVTINNFNYNDPIDNDNII**AAAAAA**ARGT	89	YTI**PP**GFNISDKNMGKEYRGQNKAINKQAYEEISKEH-------------	~~25~~90
BoNT/B	MP**A**----FNYNDPIDNDNIIMMEPPFARGT	91	YTIEEGFNISDKNMGKEYRGQNKA**AAAAAA**EEISKEHLAVYKIQMCKSVK	~~26~~92
BoNT/B	MPVTINNFN**R**----------MMEPPFARGT	93	YTIEEGFNISDKNMGKEYRGQNKAINKQAY------**AAAAAA**IQMCKSVK	~~27~~94
BoNT/C_1	M---------SDPVDNKNILYLDTHLNTLA	95	NIPKSNLNVLFMGQNLSRNPALRKVNPENML**AAA**---CHKAIDGRSLYNK	~~28~~96
BoNT/D	MT**R**PVKD----DPVNDNDILYLRIPQNKLI	97	YTIRDGFNLTNKGFNIENSGQNIERNPALQKL------DL**PP**KVCLRLTK	~~29~~98
BoNT/E	MPKINS**PP**NYNDPVNDRTILYIKPGGCQEFY	99	GYNINNLKVNFRGQNANLNPRIITPITGRGLVKK**AAAA**CKNIVSVKGIRK	~~30~~100
BoNT/E	MPKINSFNYNDP**AAAA**NDRTILYIKPGGCQEFY	101	GYNINNLKVNFRGQNANLNPRIITPITGRGLV---	~~31~~102

			HRFCKNIVSVKGIRK	
BoNT/E	MPKINSFNYNDPVNDRTIL **K**IKPGGC**K**EFY	103	GYNINNLKVNFRGQNANLN PRIITPITGRGL**PP**----- -------------	~~32~~104
BoNT/F	MP------ NYNDPVNDDTILYMQIPYE EKS	105	TVSEGFNIGNLAVNNRGQS IKLNPKIIDSIPDKG**AAAA** **AA**--CKSVIPRK	~~33~~106
BoNT/G	MPVNI**PP**---- DPINNDDIIMMEPFNDPGP	107	QNEGFNIASKNLKTEFNGQ NKAVNKEAY---------- -----**AAAAAAA**	~~34~~108

42. Please replace Table 5 with the one below:

Table 5				
Toxin ~~toxin~~	N-term (AAs 1-30) of LC	SEQ ID NO:	C-term (last 50 AAs) of LC	SEQ ID NO: ~~Seq ID #~~
BoNT/A	M--------YKDPVNGVDIAYIKIPNAGQM	109	GFNLRNTNLAANFNGQNTEINNMNFTKLKNFTGLFEFYK-----------	~~49~~110
BoNT/A	MPFVNKQ------VNGVDIAYIKIPNAGQM	111	GFNLRNTNLAANFNGQNTEINNMNFTKLK----------LLCVRGIITSK	~~50~~112
BoNT/A	MPFVNKQFNYKDP------AYIKIPNAGQM	113	GFNLRNTNLAANFNGQNTEINNMN--------GLFEFYKLLCVRGIITSK	~~51~~114
BoNT/A	MPFVNKQFNYKDPVNGVDIA----------	115	GFNLRN----------NTEINNMNFTKLKNFTGLFEFYKLLCVRGIITSK	~~52~~116
BoNT/B	MPVTINNFNYNDPIDNDNIIMME-------	117	YTI-----ISDKNMGKEYRGQNKAINKQAYEEISKEHLAVYKIQMCKSVK	~~53~~118
BoNT/B	MPVTINNFNYND----------EPPFARGT	119	YTIEEGFNISD--------GQNKAINKQAYEEISKEHLAVYKIQMCKSVK	~~54~~120
BoNT/B	MP--------NDPIDNDNIIMMEPPFARGT	121	YTIEEGFNISDKNMGKEYRGQNKAINKQA------------KIQMCKSVK	~~55~~122
BoNT/C_1	MPI-------SDPVDNKNILYLDTHLNTLA	123	NIPKSNLNVLFMGQNLSRNPALRKV----------KFCHKAIDGRSLYNK	~~56~~124
BoNT/D	MTW----------VNDNDILYLRIPQNKLI	125	YTIRDGFNLTNKGFNIENSGQNIERNPA----------DLFTKVCLRLTK	~~57~~126
BoNT/E	MP--------DPVNDRTILYIKPGGCQEFY	127	GYNINNLKVNFRGQNANLNPRIITPI----------RFCKNIVSVKGIRK	~~58~~128
BoNT/E	MPKINSFNYN---------	129	GYNINN------GQNANLNPRIITPITGRGL	~~59~~130

	-IKPGGCQEFY		VKKIIRFCKNIVSVKGIRK	
BoNT/E	MPKINSFNYNDPVNDRTIL YIK--------	131	GYNINNLKVNFRGQNANLN PRIITPITGRGLVKKIIR- -------KGIRK	~~60~~132
BoNT/F	MPVAINSFNYNDPVNDDTI LYMQIP-----	133	TVSEGFNIGNLAVNNRGQS IKLNPKIIDSIPD------ --KFCKSVIPRK	~~61~~134
BoNT/G	M------------------ -----------		QNEGFNIASKNLKTEFNGQ NKAVNKEA----------- -RIAMCKPVMYK	~~62~~135